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Award Number: W81XWH-10-1-1005

TITLE: Role of PTP1B in HER2 Signaling in Breast Cancer

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REPORT DATE: October 2012

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| October 2012 | Annual Summary | 30 September 2011 – 29 September 2012 |

**4. TITLE AND SUBTITLE**

Role of PTP1B in HER2 Signaling in Breast Cancer

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-10-1-1005

**5c. PROGRAM ELEMENT NUMBER**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

The Institute for Cancer Research
Philadelphia, PA 19111

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The receptor tyrosine kinase HER2/ ErbB2 is overexpressed in about 25% of all breast cancers, and presents an attractive therapeutic target. However, drug resistance is a significant clinical problem with current ErbB2-targeted therapies. The development of novel therapeutic strategies demands knowledge of ErbB2 receptor cross-talk with other signaling pathways. Recent reports have shown that Protein Tyrosine Phosphatase 1B (PTP1B) plays a positive role in ErbB2-induced breast cancer in vitroand in vivo. This research aims to understand the role of PTP1B-regulated pathways in ErbB2-mediated progression of breast cancer.The study uses quantitative proteomics to identify pro-oncogenic PTP1B substrates in ErbB2-transformed human mammary epithelial cells. Two cell populationsð one with normal PTP1B expression (ErbB2/PTP1B+) and the other with reduced PTP1B expression (ErbB2/PTP1B-) were subjected to SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture) coupled with quantitative phospho-proteomic analysis, which generated a list of about 1000 proteins. Some of these proteins are involved in the PTP1B mediated ErbB2-induced breast carcinogenesis, but require further validation. The ultimate aim of the present study is to identify new therapeutic targets and new biomarkers for breast cancer.

**15. SUBJECT TERMS**
SILAC, PTP1B, ErbB2, substrate trap, phosphoproteomics

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| U | U | U | UU | 7 | **19b. TELEPHONE NUMBER** *(include area code)* |

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Department of Defense, Breast Cancer Research Program (BCRP) 2009, Postdoctoral Fellowship Award

W81XWH-10-1-1005, “Role of PTP1B in HER2 Signaling in Breast Cancer"

**INTRODUCTION**

The receptor tyrosine kinase ErbB2 is overexpressed in approximately 25% of all breast cancers. ErbB2, and the signaling pathways it activates, represent potential selective targets for therapy in breast cancer. However, drug resistance is a significant clinical problem with current ErbB2-targeted breast cancer therapies. This is because of our lack of understanding of the molecular mechanism underlying the role of ErbB2 in breast carcinogenesis. Recently, Dr. Chernoff and others have reported that a cytoplasmic enzyme, protein tyrosine phosphatase 1B (PTP1B) plays a positive role in ErbB2-induced breast cancer *in vitro* and *in vivo*. Therefore, a detailed understanding of the signaling crosstalk between ErbB2 and PTP1B regulated pathways is warranted. In this research proposal, I have aimed to identify key pro-oncogenic PTP1B regulated pathways in ErbB2 signaling in MCF10A breast epithelial cells using quantitative phospho-proteomics. This study has the potential to uncover novel molecular targets in ErbB2-positive breast cancer and is expected to provide new hope and direction to the present breast cancer biomarkers and therapeutics.

**BODY**

In the approved Statement of Work, my first goal was to identify global PTP1B regulated pathways in ErbB2 signaling in MCF10A breast epithelial cells using SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture). SILAC, developed by Mann and colleagues, is an *in vivo* labeling approach where the proteome is labeled as the cells grow in culture [1] and can be used to compare relative changes in protein phosphorylation levels between samples [2]. This task had been projected to be completed within a timeframe of approximately 18 months. This has been accomplished and the following actions as outlined in the Statement of Work were executed successfully:

i. PTP1B was knocked down in 10A.ErbB1:B2 cells [3] (MCF10A cells expressing a chimeric, rapalog-stimulatable form of ErbB1:ErbB2) by stable transfection with a PTP1B specific siRNA expression construct [4]. Stable transfection of 10A.ErbB1:B2 cells with a scrambled siRNA construct was used as the control. Knockdown of PTP1B was confirmed to be >75% in the appropriate cell line by assessing PTP1B levels by Western Blots (Figure 1).

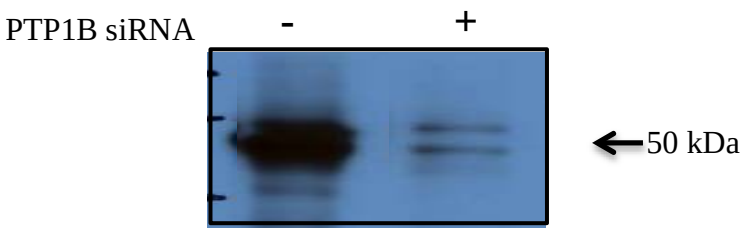


**Figure 1:** Western Blot analysis of 10A.ErbB1:B2 whole cell lysates with PTP1B monoclonal antibody.

ii. The cell population with normal PTP1B expression (ErbB1:B2/PTP1B+) was labeled with media containing heavy isotopes of Lys and Arg (C13 labeled Lys and C13 labeled Arg). The cell population with reduced PTP1B expression (ErbB1:B2/PTP1B-) was labeled with media containing light or normal isotopes of Lys and Arg. After labeling of the two cell populations with appropriate SILAC media, the chimeric ErbB1:ErbB2 in the two populations was activated with the hetero-dimerizing agent, rapalog.

iii. The lysates from the differentially labeled cells (ErbB1:B2/PTP1B+ and ErbB1:B2/PTP1B-) were pooled and the phosphotyrosyl proteins were immunoprecipitated with agarose-conjugated antiphosphotyrosine 4G10 Platinum monoclonal antibody from Millipore. The phosphotyrosyl proteins were eluted using phenyl phosphate, followed by SDS-PAGE analysis.

iv. Protein bands were cut out for trypsin digestion; and the phosphotyrosyl tryptic peptides analyzed using LC-MS/MS with the help of Dr. Steven Seeholzer, Director of the Protein Core Facility at the Childeren's Hospital of Philadelphia (CHOP), who has expertise with various proteomic techniques. Analysis of mass spectra results using MASCOT and peptide quantification using MSQUANT open source software [5].

Earlier studies on ErbB2 signaling in NIH3T3 fibroblasts using similar approach identified about 200 proteins including previously known as well as unknown proteins [6]. A similar number were identified in SILAC study using PTP1B wild type and knock out mouse embryonic fibroblasts [7]. The present study generated a list of about 1,000 proteins. The list of proteins is available upon request via email: Sayanti.Saha@fccc.edu

My next goal was to use substrate trapping [8] to distinguish between direct and indirect PTP1B substrates. This task had been projected to be completed in the second year of the study. The following actions were executed:

i. A GST-tagged substrate trap PTP1B plasmid (GST-PTP1B$_{D181A/Q262A}$) was generated by site-directed mutagenesis using the GST-tagged wild type PTP1B plasmid as the template. The GST-fusion proteins were expressed in bacteria and purified using standard methods.

ii. ErbB1:B2/PTP1B- cells were treated with the hetero-dimerizing agent, rapalog to activate the chimeric ErbB1:ErbB2, following which the cells were lysed on ice in lysis buffer (20mM Tris, pH 7.5, 100mM NaCl, 1% Triton X-100, 10% glycerol, 5 mM iodoacetic acid, 1 mM orthovanadate, and proteases inhibitors). The lysis buffer contains iodoacetic acid to irreversibly inactivate the endogenous protein tyrosine phosphatases. Dithiothreitol (10mM final) was then added for 15 min at 4 °C to inactivate any unreacted iodoacetic acid. The cell lysate was centrifuged at 14,000g for 15min, and the cleared lysates were incubated in two separate tubes with GST–PTP1B$_{WT}$ and GST-PTP1B$_{D181A/Q262A}$ conjugated to glutathione–Sepharose beads overnight at 4°C.

iii. After incubation, the beads were washed to remove any nonspecific proteins; resuspended in Laemmli sample buffer and sent for proteomic analysis to Protein Core Facility at the Childeren's Hospital of Philadelphia (CHOP).

The result obtained from the analysis was corrupted since no protein peak corresponding to the mutant PTP1B$_{D181A/Q262A}$ was seen, and it was not possible to proceed further with the obtained data.

## KEY RESEARCH ACCOMPLISHMENTS

- A stable knockdown of PTP1B was generated in 10A.ErbB1:B2 cells and confirmed by Western Blot analysis.
- SILAC study was performed for the very first time in mammary epithelial cells, which revealed about 1000 proteins which could potentially be involved in PTP1B regulated pathways. However, this requires further validation.

## REPORTABLE OUTCOMES

- A stable knockdown of PTP1B was generated in 10A.ErbB1:B2 cells
- The work done so far was presented as a poster at the 2011 Era of Hope Conference on Breast Cancer held in Orlando from Aug 2-5, 2011
- The present work resulted in a database of proteins which could potentially be regulated by PTP1B in response to ErbB2 signaling in mammary epithelial cells

## CONCLUSIONS

This research project aims to enhance our knowledge database about the role of PTP1B in oncogenic signal transduction, with specific focus on breast cancer. An understanding of the detailed molecular mechanism by which PTP1B regulates ErbB2-induced breast carcinogenesis is expected to provide new hope and direction to the present breast cancer therapeutics. An in depth understanding of the molecular pathways leading to the development of breast cancer (as was expected from the present study) can help in the identification of new biomarkers for breast cancer, in addition to identifying new targets for breast cancer therapeutics.

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## BIBLIOGRAPHY OF PUBLICATIONS

None

## LIST OF PERSONNEL

Sayanti Saha, Ph.D. – Principal Investigator

## APPENDIX

None